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SEPARATION OF PROTEINS IN MOLLUSC SHELLS BY GEL-FILTRATION

Proteins and glycoproteins are involved in biological mineralization processes. For example, collagens in bone structures of vertebrates promote the formation of apatite seeds^{1/}, whereas glycoproteins in the exoskeleton of crabs provide a set of highly specific templates for calcite nucleation^{2/}. In molluscs, a wide array of structurally different proteins are contained in the shell structures, attesting to the heterogeneity of calcified tissues^{3/}.

It has been suggested that independent of the kind of protein participating in crystal-seed formation, the most essential factor, in nucleating a mineral phase appears to be the availability of free carboxyl groups provided by certain acidic amino acids and free amino groups by certain basic amino acids and hexosamines^{1-4/}. It is thus inferred that throughout the animal kingdom, Nature adheres to the same principles when it comes to the formation of mineral nuclei.

To test this assumption, mollusc shell tissues of widely different biochemical composition and phylogeny have been studied by means of enzymatic and non-enzymatic degradation techniques and by gel-filtration. Tryptic digests did not result in the dissolution of shell matrix proteins but treatments with urea, hydroxylamine and formic acid degraded the tissues. According to preliminary studies, the hydroxylamine treatment resulted in a cleavage of the protein molecule to units of molecular weight of approximately 20,000 to 80,000^{4/}.

The analytical scheme followed during the present investigation is outlined in Figure 1. The decalcified organic tissue was treated with a

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hydroxylamine/6 M Urea solution for 24 hours at 45°C and subsequently with 95% formic acid for 4 hours at 45°C. Each treatment was followed by centrifugation and by gel-filtration of the supernatant, on G-25 Sephadex columns.

A variety of buffer systems were tested and the molality of the buffers (0.1 to 1) did not play a significant role in the efficiency of separation. Although some buffer systems proved to be slightly better than the routinely used borax buffer of pH 9 (hydroxylamine/urea fraction) and formic acid buffer of pH 3.0 (formic acid fraction) the two latter ones interfered least with the subsequent amino acid analysis. The high-molecular fractions (M.W.>5.000) were hydrolyzed and examined for their amino acid composition^{4/}. To facilitate a comparison, the amino acid analysis of the original samples and the residues are included in Table I.

The analytical data indicate that throughout the molluscan phylum the urea/hydroxylamine fraction is consistently enriched in aspartic acid, lysine and amino sugars although this relationship is not necessarily displayed when these three amino compounds are highly concentrated in the original shell. It is tentatively suggested that this peptide fraction contains the active sites for the deposition of the mineral phase and that the remaining part of the proteinaceous matrix in the shells is not involved in the actual calcification. It is reasonable to assume that similar structures are contained in other mineralized tissues such as are found in bones or teeth. A full account on the phylogenetic and chemical aspects of these experiments will be presented elsewhere, together with several hundred amino acid analyses from calcified and uncalcified proteins^{5/}.

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Figure 1 Flow diagram of analytical procedures

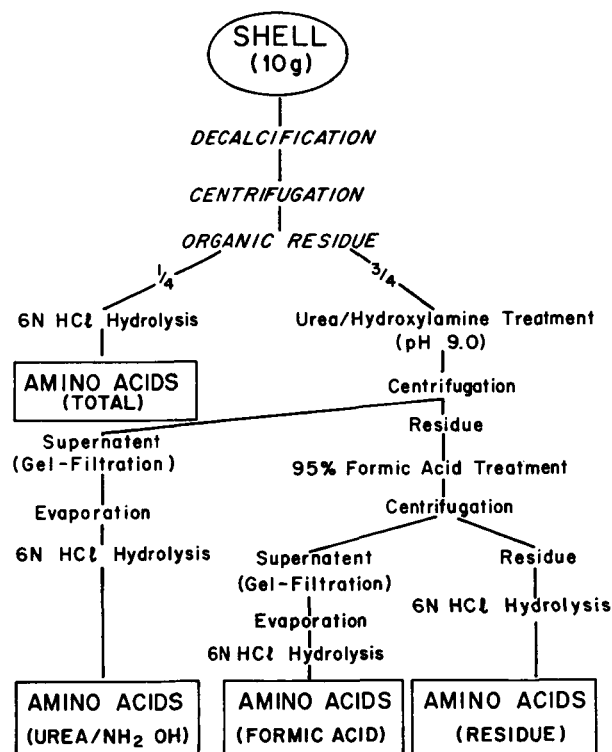


TABLE 1 A

AMINO ACID COMPOSITION OF MOLLUSC SHELL PROTEINS AND ISOLATED
PEPTIDE/PROTEIN FRACTIONS - (IN RESIDUES PER 1000)

	CRYPTOCHITON				NAUTILUS				HALIOTIS				ACHATINELLA			
	TOTAL		UREA/ NH ₂ OH		FORMIC ACID		RESIDUE		TOTAL		UREA/ NH ₂ OH		FORMIC ACID		TOTAL	
	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(3)	(2)	(3)	(1)	(2)	(1)	(4)	(2)
ASPARTIC ACID	117	118	118	112	89	70	85	70	186	160	186	160	185	36	64	19
THREONINE	58	62	68	71	38	23	19	23	43	32	43	32	53	12	23	8
SERINE	75	49	68	77	55	109	113	109	101	144	101	144	79	53	35	8
GLUTAMIC ACID	103	103	110	111	56	59	52	59	50	32	50	32	131	27	42	11
PROLINE	105	136	144	120	53	5	10	5	36	37	36	37	52	63	61	50
GLYCINE	128	122	139	101	270	255	296	255	212	350	212	350	156	417	365	520
ALANINE	100	75	82	86	69	248	223	248	163	63	163	63	86	53	40	24
CYSTINE	19	12	18	25	21	7	9	7	3	-	3	-	1	18	3	11
VALINE	50	51	50	65	23	20	16	20	22	21	22	21	53	92	69	52
METHIONINE	16	15	12	25	1	5	5	5	4	2	4	2	3	1	5	1
ISOLEUCINE	31	33	32	30	16	18	15	18	12	12	12	12	32	63	58	39
LEUCINE	52	46	43	68	24	27	27	27	29	27	29	27	68	75	99	108
TYROSINE	23	19	16	16	38	9	13	9	20	6	20	6	10	14	13	17
PHENYLALANINE	39	38	32	29	58	61	59	61	31	29	31	29	36	59	64	89
OH-LYSINE	-	-	-	-	1	1	1	1	1	1	1	1	-	-	-	-
LYSINE	24	28	15	16	28	4	3	4	19	45	19	45	51	12	41	22
HISTIDINE	6	18	10	14	14	2	3	2	3	1	3	1	2	2	3	2
ARGININE	54	75	43	34	22	77	51	77	65	38	65	38	2	3	15	19
PROTEIN																
HEXOSAMINES	1.11	7.12	7.6	0.87	43	661	121	661	33	35	33	35	67	302	92	89
PER CENT PROTEIN IN TOTAL SHELL	2.87				2.24		2.24		1.12		1.12		0.68			

* Number in parentheses indicate the number of individual analyses.

TABLE 1 B

AMINO ACID COMPOSITION OF MOLLUSC SHELL PROTEINS AND ISOLATED
PEPTIDE/PROTEIN FRACTIONS - (IN RESIDUES PER 1000)

	MYTILUS			AEQUIPECTEN			MERCENARIA			LAEVICARDIUM		
	TOTAL	UREA/ NH ₂ OH	FORMIC ACID	TOTAL	UREA/ NH ₂ OH	FORMIC ACID	TOTAL	UREA/ NH ₂ OH	FORMIC ACID	TOTAL	UREA/ NH ₂ OH	FORMIC ACID
	(5)	(2)	(1)	(4)	(1)	(1)	(4)	(3)	(1)	(4)	(1)	(1)
ASPARTIC ACID	95	159	118	296	340	245	148	196	187	125	141	101
THREONINE	19	56	17	36	15	30	47	54	47	57	28	73
SERINE	93	86	98	186	218	143	75	102	52	58	127	69
GLUTAMIC ACID	36	84	41	59	57	63	71	84	95	90	53	106
PROLINE	20	82	15	23	21	36	128	91	126	87	66	67
GLYCINE	357	226	293	214	211	228	146	103	103	195	227	138
ALANINE	183	84	216	62	44	55	70	57	77	68	81	45
CYSTINE	13	13	13	12	9	20	21	24	12	90	97	99
VALINE	27	36	29	18	15	37	32	35	35	56	16	78
METHIONINE	7	9	6	6	1	1	14	12	3	25	1	8
ISOLEUCINE	15	24	18	12	8	21	24	22	22	31	6	44
LEUCINE	49	52	48	25	19	31	35	35	46	44	54	74
TYROSINE	34	13	24	5	4	12	42	41	34	17	15	10
PHENYLALANINE	15	16	17	8	6	13	33	29	35	20	29	11
OH - LYSINE				1	1	1						
LYSINE	14	46	18	26	23	43	68	69	75	24	31	41
HISTIDINE	4	2	5	3	5	9	5	22	9	5	16	10
ARGININE	19	12	24	8	3	12	41	24	42	10	12	28
PROTEIN												
HEXOSAMINES	394	37	856	118	61	n.d.	102	45	85	179	48	250
PERCENT PROTEIN IN TOTAL SHELL	0.91			0.25			0.28			0.54		

* Number in parentheses indicate the number of individual analyses.